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(71) Applicant *(for all designated States except US)*: CIBA-GEIGY AG [CH/CH]; Klybeckstrasse 141, CH-4002 Basle (CH).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: REED, Kenneth, W. [US/US]; 1241 Fairfax Hunt, Lawrenceville, GA 30243 (US). YEN, Shau-Fong [US/US]; 1295 North Druid Hills Road, Atlanta, GA 30319 (US).

(74) Common Representative: CIBA-GEIGY AG; Patentabteilung, Klybeckstrasse 141, CH-4002 Basle (CH).

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(54) Title: COMPOSITIONS INCLUDING O-CARBOXYALKYL CHITOSAN AND METHODS OF USE IN OPHTHALMICS

(57) Abstract

Compositions including O-carboxyalkyl chitosan and use of said compositions in ophthalmic formulations. O-carboxyalkyl chitosan enhances ocular bioavailability and is especially useful in ophthalmic compositions which must be held at an acidic pH for storage, and which must remain clear when applied to the eye at a physiological pH of about 7.4.

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COMPOSITIONS INCLUDING O-CARBOXYALKYL CHITOSAN AND METHODS OF USE IN OPHTHALMICS

FIELD OF THE INVENTION

This invention relates broadly to compositions including O-carboxyalkyl chitosan and methods of use. More specifically, the invention relates to O-carboxyalkyl chitosan compositions useful in ophthalmic applications.

DESCRIPTION OF THE RELATED ART

Chitin, poly(N-acetyl-D-glucosamine), is a naturally occurring substance found in shellfish. Chitosan is a partially deacetylated derivative of chitin. More specifically, chitosan is a polysaccharide which consists of N-acetyl-D-glucosamine and D-glucosamine units linked together by $\beta(1\rightarrow4)$ glycosidic bonds. The "degree of deacetylation" in a sample of chitosan refers to the relative amounts of the deacetylated and acetylated monosaccharides present in the chitosan sample. The preparation of chitosan is disclosed in U.S. Patent No. 2,040,880, issued on May 19, 1936.

N,O-carboxyalkyl chitosans are derivatives of chitosan formed by carboxyalkylation of chitosan. The carboxyalkyl groups of N,O-carboxyalkyl chitosan are located at the primary amino group on the D-glycosamine group and at the hydroxyl groups. N,O-carboxymethyl chitosan is water soluble and may be formed by carboxymethylation of chitosan. N,O-carboxymethyl chitosan is commercially available from NOVACHEM, Halifax, N.S., Canada. The preparation of N,O-carboxymethyl chitosan is disclosed in U.S. Patent No. 4,619,995, issued on Oct. 28, 1986, to E. Hayes.

Various uses of chitin and chitosan have been disclosed in the art. For example, P. Sandford, et al., "Biomedical Applications of High Purity Chitosan", Ch. 28, Water-Soluble Polymers, discloses various properties and uses of chitosan.

U.S. Patent No. 4,365,050, issued to Ivani on Dec. 21, 1982, discloses ophthalmic wetting solutions and viscosity builders. These ophthalmic compositions include aminopolysaccharides, primarily N-acetyl-D-glucosamines and derivatives. U.S. Patent No. 4,447,562, issued on May 8, 1984, also to Ivani, discloses pharmaceutical compositions including aminopolysaccharides.

European Patent Application No. 0 426 368 A2, P. Highan, et al., discloses the use of cross-linked biodegradable derivatives of chitin for use in preventing adhesion between body tissues. The preferred chitin derivative is N,O-carboxymethyl-chitosan, but O-carboxymethyl-chitosan is also suggested as useful in preventing adhesion. In contrast to N,O-carboxyalkyl chitosan, the O-carboxyalkyl derivative of chitosan, has the carboxyalkyl group attached to a free hydroxyl group (typically at the 6 position) of some of the chitosan monosaccharides groups.

SUMMARY OF THE INVENTION

An object of the invention is to provide compositions suited for use as excipients in ophthalmic formulations to improve ocular retention and ocular bioavailability.

Another object of the invention is to provide an ophthalmic retention-enhancing material which may be formulated and/or maintained at an acidic pH without excessive turbidity and phase separation.

One embodiment of the invention is an ophthalmic composition including O-carboxyalkyl chitosan. In a preferred embodiment, the composition includes a delivery agent which is chemically sensitive to pH, and the pH of the composition is held at an acidic level to enhance stability of the delivery agent.

Another embodiment of the invention is a method of delivering an agent to the ocular environment, which method includes providing an ophthalmic composition including O-carboxymethyl chitosan at a pH of about 4 to 6, and dispensing the ophthalmic composition to the ocular environment through a means for altering the pH, thereby altering the pH to an ocularly acceptable pH immediately before ocular administration.

Yet another embodiment of the invention is an ophthalmic dispenser including a container defining a reservoir and having an outlet; an ophthalmic composition including O-carboxymethyl chitosan at a pH of about 4 to 6, retained within the reservoir; and pH-altering means for increasing the pH of the composition, with the pH-altering means being positioned in fluid communication between the solution and the dispenser outlet. In operation, the pH of the acidic composition is increased by passing the composition through the pH-altering means when administering the composition to the eye.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 is a graph of percent miosis v. minutes after instillation for 2% pilocarpine solutions, comparing N,O-carboxymethyl chitosan and hydroxypropylmethyl cellulose.

FIG. 2 is a graph of percent miosis v. minutes after instillation for a 1% pilocarpine solution with hydroxypropylmethyl cellulose and a 0.5% pilocarpine solution with O-carboxymethyl chitosans having three different degrees of carboxymethylation.

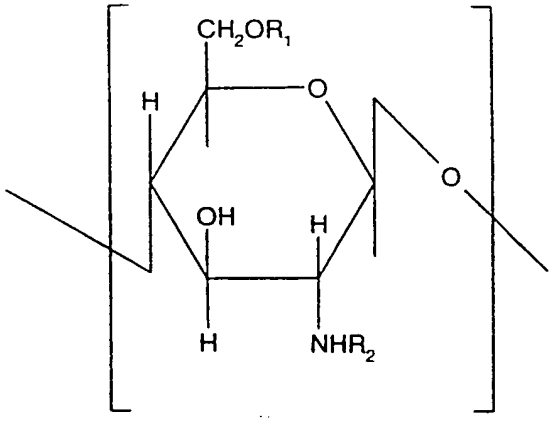
FIG. 3 is a graph of percent miosis v. minutes after instillation for 0.5% pilocarpine solutions with chitosan having a 20 minute carboxymethylation time, comparing autoclaved to non-autoclaved samples.

FIG. 4 is a graph of percent miosis v. minutes after instillation for 0.5% pilocarpine solutions with chitosan having a 180 minute carboxymethylation time, comparing autoclaved to non-autoclaved samples.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

One embodiment of the present invention is an O-carboxyalkyl chitosan-containing composition for use in ophthalmic products. Ophthalmic products include a wide range of products intended for intimate contact with the ocular environment, i.e., eye tissue, ocular surrounding fluids, or tissue surrounding the eye. O-carboxyalkyl chitosan may be used to improve ocular retention and ocular bioavailability in ophthalmic solutions. Further, O-carboxyalkyl chitosan may be used in enteric-coated agent delivery devices, which are those devices which provide some control in the delivery of an agent to the gastrointestinal tract. The term "agent", as used herein, means drug, pharmaceutical, diagostic agent, vitamin, or other agent which is advantageous to delivery to the ocular environment.

O-carboxyalkyl chitosan, as used herein, is defined as follows:



where R_1 is H about 0 to 82% of the time and carboxyalkyl about 18-100% of the time, and where R_2 is H about 50 to 100% of the time and acetyl about 0 to 50% of the time. The O at the 3-position may be substituted with R_1 some of the time, but the species shown is the predominate species.

OPHTHALMIC COMPOSITIONS AND METHODS

It has been discovered that O-carboxyalkyl chitosan has certain properties which may be advantageously used in ophthalmic products or in enteric coatings. One advantage of O-carboxyalkyl chitosan relates to retention-enhancing or bioavailability-enhancing characteristics, while the other relates to the pH of the formulation. First, it has been discovered that O-carboxyalkyl chitosan provides better bioavailability of delivery agents than does N,O-carboxyalkyl chitosan. Thus, O-carboxyalkyl chitosan compositions have demonstrated unexpected advantages over N,O-carboxyalkyl chitosan compositions in enhancing ocular retention or bioavaibility of ophthalmic delivery agents.

A second advantage of O-carboxyalkyl chitosan compositions relates to the ability to maintain and/or formulate a delivery composition at an acidic or neutral pH. Chitosan precipitates out of solution when adjusted to a physiological pH of about 7.4, which is the pH of human tear fluid. Precipitation of an ophthalmic retention-enhancing agent upon contact with the eye may cause blurred vision. In contrast to chitosan, O-carboxyalkyl chitosan, which has water soluble carboxyalkyl groups substituted at the O-position, has been found to remain soluble at the pH of the tear fluid. Similarly, N,O-carboxymethyl chitosan is also soluble in tear (ocular) fluid at physiological pH. Thus, neither O-carboxy-alkyl chitosan nor N,O-carboxyalkyl chitosan will precipitate out when applied to the eye.

However, N,O-carboxymethyl chitosan cannot be formulated at a pH much lower than physiological pH (i.e., pH = 7.4). Turbidity and phase separation occurs in N,O-carboxymethyl chitosan-containing compositions at a pH of about 6.6 or lower. Although a physiological pH of 7.4 is preferred for application to the eye in order to maximize patient comfort, many active agents require a lower pH in order to avoid stability problems (i.e., to enhance shelf life). Thus, while it is commonly accepted that a pH of about 6 to 8 is comfortable to the eye, stability problems require that some commercial formulations maintain pH values as low as about 3. For example, pilocarpine formulations are typically held at a pH of about 5 or lower.

While compositions may be formulated with N,O-carboxymethyl chitosan at a pH lower than 6.6, the phase separation requires the consumer to agitate the composition before use. Also, immediately subsequent to application to the eye, the consumer will experience blurred vision because of the turbidity. Further, turbid ophthalmic compositions are not aesthetically appealing to the consumer.

In contrast, O-carboxyalkyl chitosan may be formulated at a pH of about 4 or higher without precipitation out of solution. Thus, preferably, the ophthalmic formulation has a pH of about 4 or higher, more preferably about 4.5 or higher. In a preferred embodiment, the ophthalmic solution has a pH of about 4 to 9. In another preferred embodiment, the ophthalmic solution has a pH of about 4 to 6. A preferred O-carboxyalkyl chitosan is O-carboxymethyl chitosan. O-carboxymethyl chitosan is commercially available from Nova Chem., Ltd., Halifax, N.S., Canada.

The O-carboxyalkyl chitosan molecular weight may range from about 1000 Daltons to about 5,000,000 Daltons, depending on the intended use of the final product. The compositions of the present invention may have a viscosity of about 1 to 200,000 centipoise at 25°C, depending again on the intended use of the product. A preferred viscosity range for an ophthalmic product which delivers an agent to the ocular environment is about 50 to 100,000 centipoise. A preferred viscosity range for an artificial tears product is about 50 to 500 centipoise.

The present O-carboxyalkyl chitosan ophthalmic compositions are especially advantageous in delivery of agents to the ocular environment, i.e., to the tear fluid, eye, or surrounding ocular tissues. A wide variety of agents may be delivered in accordance with

the present invention, including, without limitation, beneficial pharmaceutical agents, diagnostic agents, vitamins, nutrients, lubricants, and the like. The ophthalmic delivery agent may include, without limitation thereto, 3H-thymidine, acetylcholine chloride, acyclovir, adrenaline, amethocaine, aminocaproic acid, antazoline phosphate, arachidonic acid, atropine, benoxinate hydrochloride, betaxolol hydrochloride, bupivacaine, carbachol, carteolol, chloramphenicol, chlortetracycline hydrochloride, chymatrypsin, clonidine, cocaine, corynanthine, cromolyn sodium, cyclopentolate, demecarium bromide, dexamethasone, dibutoline, dichlorphenamide, diclofenac, dipivefrin hydrochloride, echodtiophate iodide, ephedrine, epinephrine bitartrate, erythromycin, ethambutol, etidocaine, eucatropine, fluoromethalone, fluorometholone, gentamicin sulfate, gramicidine, H-thymidine, homatropine hydrobromide, hyaluronic acid, hydrocortisone, idoxuridine, indomethacin, inositol triphosphate, inositol phosphates, isoflurophate, isosorbide, lachesine, levobunolol, levocabastine, lidocaine, lignocaine, medrysone, mepivacaine, methacholine, methazolamide, naphazoline hydrochloride, natamycin, neomycin sulfate, neostigmine, noradrenaline, ofloxacin, oxybuprocaine, oxymetazolin, oxyphenonium, pheniramine maleate, phenylephrine hydrochloride, phosphatidylinositol phosphates, physostigmine, pilocarpine hydrochloride, polyhexamethylene biguanides, polymyxin B sulfates, prednisolone sodium phosphate, proparacaine hydrochloride, proxymethacaine, pyrilamine maleate, scopolamine hydrobromide, sorbinil, sulfacetamide, sulfisoxazole disolamine, tamoxifen, tetracaine hydrochloride, tetracycline, tetrahydrozoline hydrochloride, timolol maleate and hemihydrate, trifluridine, tropicamide, vidarabine, and other ophthalmically acceptable salts thereof and mixtures thereof.

One preferred set of delivery agents includes those which degrade during storage in solutions at a pH which is not acidic, i.e., "pH-sensitive delivery agents". Thus, one preferred group of ophthalmic delivery agents includes pilocarpine, epinephrine, dipivefrin, hydralazine, carbachol, ophthalmically acceptable salts thereof and mixtures thereof. Particularly preferred delivery agents include hydralazine and pilocarpine, and ophthalmically accceptable salts thereof.

The ophthalmic compositions of the present invention include about 0.1 to about 25 weight percent O-carboxyalkyl chitosan. More preferably, the ophthalmic product includes about 1 to about 10 weight percent O-carboxyalkyl chitosan.

In a preferred embodiment, the ophthalmic compositions include (a) about 0.01 to 10 weight percent of a delivery agent; (b) about 0.1 to 25 weight percent O-carboxyalkyl

chitosan; and (c) about 99.89 to 74.99 weight percent ophthalmic carrier. A more preferred ophthalmic composition includes (a) about 0.01 to 2.0 weight percent of a delivery agent; (b) about 1 to 6 weight percent O-carboxyalkyl chitosan; and (c) about 98.99 to 93.99 weight percent ophthalmic carrier.

Ophthalmic carrriers may be chosen from a wide variety of carriers known in the art which are ophthalmically acceptable. Ophthalmic carriers include, without limitation thereto, water, petrolatum, mineral oil, silicone oil, and natural vegetable oils such as olive oil.

In a preferred embodiment, the O-carboxyalkyl chitosan (or composition containing O-carboxyalkyl chitosan) is subjected to autoclaving. Autoclaving at elevated temperatures is useful for sterilizing ophthalmic compositions. However, a disadvantage of autoclaving, for some ophthalmic compositions, is that the ocular retention-enhancing component may be degraded, decomposed, or otherwise damaged. Damage to the retention-enhancing component(s) ultimately reduces the bioavailability of the delivery agent upon application to the eye. However, it has been surprisingly found that autoclaving O-carboxyalkyl chitosan can improve the ocular retention-enhancing characteristics. This increase in retention-enhancing characteristics is more pronounced with higher levels of carboxyalkylation at the O-position. The temperature and duration of autoclaving may vary depending on the specific composition and application, but a temperature of about 100 to 150°C for a period of about 5 to 60 minutes is a useful range. Thus, in a preferred embodiment, the O-carboxyalkyl chitosan, or composition thereof, is autoclaved at elevated temperatures.

The O-carboxyalkyl chitosan-containing ophthalmic compositions which are prepared at an acidic pH for storage may be altered to increase the pH immediately prior to administration of the solution to the ocular environment. It is generally accepted that a pH of 6 to 8 does not produce patient discomfort, i.e., a pH of 6 to 8 is ocularly compatible. Thus, in a preferred embodiment, a method of delivering an agent to the ocular environment is provided, which method includes the providing an ophthalmic composition including O-carboxyalkyl chitosan at an acidic pH, preferably above about 4, more preferably about 4 to less than about 6, and dispensing said ophthalmic composition to the ocular environment through a means for altering the pH, thereby altering the pH to an ocularly acceptable pH.

There are numerous methods of increasing the pH of the ophthalmic solution immediately prior to contact with the eye. For example, U.S. Patent Nos. 5,056,689 and 5,080,800, issued to Heyl, et al., disclose ophthalmic dispensers including scavenger media

positioned between the solution and the dispenser outlet. In operation, the media removes a component of the solution when the consumer passes the solution through the media while dispensing the solution to the eye. In the case of low pH solutions, a media which increases the solution pH upon contact may be provided in the dispenser tip. U.S. Patent Nos. 5,056,689 and 5,080,800 are incorporated herein by reference. Accordingly, a particularly suitable scavenger media is preferably selected from a negatively and/or a positively charged scavenging material, e.g. an ion exchange resin. A particular example is a scavenging material comprised of a mixture of "Bio Rex 5" and "AG-4", both Bio Rad products, in a 75 to 25 ratio, which will almost completely remove 0.1% sorbic acid from an aqueous solution and raise the pH of the solution from 4.0 to about 7.0.

Thus, another embodiment of the invention is an ophthalmic dispenser including (a) a container defining a reservoir and having an outlet; (b) an ophthalmic composition. including O-carboxymethyl chitosan at a pH of about 4 to 6, retained within the reservoir; and (c) pH-altering means for increasing the pH of the composition, with the pH-altering means being positioned in fluid communication between the solution and the dispenser outlet.

The previous disclosure will enable one having ordinary skill in the art to practice the invention. In order to better enable the reader to understand specific embodiments and the advantages thereof, reference to the following examples is suggested.

EXAMPLE I

An aqueous solution containing 2 weight percent pilocarpine and about four (4) weight percent N,O-carboxymethyl chitosan is prepared as follows. About 5 grams glacial acetic acid and about 6 grams sodium chloride is added to about 900 ml of deionized water. About 40 grams of N,O-carboxymethyl chitosan (Protan Laboratories, Redmond, VA) is dissolved in the aqueous solution. About 20 grams of pilocarpine (Sigma Chemical Co.) is added to the solution, with the pH being adjusted to about 5 by adding 1N HCl. The final volume is adjusted q.s. to 1 liter.

The resultant solution is evaluated by measuring the pupil diamter of rabbit eyes at various times after instillation of 30 microliters of the solution into the rabbit eye, and calculating miotic effect from the measured pupil diameters. Miosis is a relative measure of pupil constriction. Administration of pilocarpine to the eye causes the pupil to constrict. Thus, the effectiveness of a pilocarpine delivery solution may be expressed by "percent miosis". "Percent miosis", as used herein, is defined by the following equation:

$$\text{percent miosis} = (D_{new} - D_{base}) / D_{base} \cdot 100$$

where D_{base} = baseline pupil diameter (prior to solution contact)

D_{new} = new pupil diameter after a given contact time with the test solution

Averaged percent miosis as a function of time from instillation into the eye is presented in TABLE 1.

EXAMPLE II

An aqueous solution containing 2 weight percent pilocarpine and 4.5 mg/ml hydroxypropyl methylcellulose (HPMC) is evaluated. The solution is a SPERSACARPINE™ solution, which is commercially available from CIBA Vision, AG (Dispersa, AG), Hettlingen, Switzerland.

TABLE 1 gives the averaged percent miosis as a function of time from instillation into the eye. FIG.1 graphically illustrates the data of TABLE 1.

TABLE 1

Time (minutes) from instillation in rabbit eye	Percent Miosis for 2% pilocarpine with 4.5 mg/ml HPMC (SPERSACARPINE™)	Percent Miosis for 2% pilocarpine in 4% N,O-carboxymethyl chitosan
0	0.0	0.0
20	47.0	40.7
30	44.9	36.4
40	41.0	29.1
60	32.4	26.5
120	25.0	14.5
180	10.4	8.7
240	6.0	1.8

EXAMPLES I and II and FIG. 1 illustrate that 4.5 mg/ml hydroxypropylmethyl cellulose performs similarly to 4 weight percent N,O-carboxylmethyl chitosan in miosis profile as a

function of time. However, HPMC performs slightly better than N,O-carboxymethyl chitosan with pilocarpine, as indicated by magnitude of miosis at a given time.

EXAMPLE III

The procedures outlined in EXAMPLE I are used to test a SPERSACARPINE™ solution having one (1) weight percent pilocarpine and 4.5 mg/ml HPMC. The data is presented in TABLE 2 and FIG. 2.

EXAMPLE IV

About 3 grams of 180-minute carboxymethylated O-carboxymethyl chitosan (Nova Chem., Ltd.) is dissolved in about 80 ml of distilled water. pH is adjusted to about 12 with sodium hydroxide. About 1.6 grams mannitol is added to adjust osmolality to near isotonic. The solution is autoclaved in a Yamato SM32 autoclave at about 121°C for about 15 minutes. The pH is adjusted to about 5 with concentrated HCl. About 0.5 grams pilocarpine hydrochloride is added to the solution. The volume is adjusted q.s. to 100 ml.

The resulting solution, including about 0.5 weight percent pilocarpine in three (3) weight percent of 180-minute-carboxymethylated O-carboxymethyl chitosan, is evaluated as outlined in EXAMPLE I. The data is presented in TABLE 2 and FIG. 2.

EXAMPLE V

About 1.5 grams of 20-minute carboxymethylated O-carboxymethyl chitosan (Nova Chem., Ltd.) is dissolved in about 40 ml of distilled water. The pH is adjusted to about 12 with sodium hydroxide. About 0.9 grams mannitol is added to adjust the osmolality to near isotonic. The solution is autoclaved in a Yamato SM32 autoclave at about 121°C for about 15 minutes. The pH is adjusted to about 5 with concentrated HCl. About 0.25 grams pilocarpine hydrochloride is added to the solution. The volume is adjusted q.s. to 50 ml.

The resulting solution, including about 0.5 weight percent pilocarpine in three (3) weight percent of 20-minute-carboxymethylated O-carboxymethyl chitosan, is evaluated as outlined in EXAMPLE I. The data is presented in TABLE 2 and FIG. 2.

EXAMPLE VI

About 1.5 grams of 60-minute carboxymethylated O-carboxymethyl chitosan (Nova Chem., Ltd.) is dissolved in about 40 ml of distilled water. The pH is adjusted to about 12 with sodium hydroxide. About 0.8 grams mannitol is added to adjust osmolality to near isotonic. The solution is autoclaved in a Yamato SM32 autoclave at about 121°C for about 15 minutes. The pH is adjusted to about 5 with concentrated HCl. About 0.25 grams pilocarpine hydrochloride is added to the solution. The volume is adjusted q.s. to 50 ml.

The resulting solution, about 0.5 weight percent pilocarpine in three (3) weight percent of 60-minute-carboxymethylated O-carboxymethyl chitosan, is evaluated as outlined in EXAMPLE I. The data is presented in TABLE 2 and FIG. 2.

TABLE 2

Time (minutes) from instillation in rabbit eye	Percent miosis			
		Autoclaved		
	1% pilocarpine with 4.5 mg/ml HPMC (SPERSACARPINE™)	0.5% pilocarpine in 3% of 20-min.-CM O-CM chitosan	0.5% pilocarpine in 3% of 60-min.-CM O-CM chitosan	0.5% pilocarpine in 3% of 180-min.-CM O-CM chitosan
0	0.0	0.0	0.0	0.0
20	32.8			
25		27.7	37.1	45.1
30	27.3			
40	21.8	30.7	34.6	43.8
60	16.3	24.4	32.1	42.9
120	8.0	17.2	23.3	33.8
180	3.7	7.6	13.3	31.1
240	0.0	0.0	3.3	26.0

EXAMPLES III-VI and FIG. 2 illustrate that a lower pilocarpine concentration (0.5%) in O-carboxylmethyl chitosan performs more effectively than a higher pilocarpine concentration (1%) in 4.5 mg/ml hydroxypropylmethyl cellulose. Thus, O-carboxymethyl

chitosan performs more effectively as an ophthalmic drug delivery vehicle than HPMC or N,O-carboxymethyl chitosan. EXAMPLES IV-VI and FIG. 2 also illustrate that the effectiveness of O-carboxylmethyl chitosan increases with time of carboxymethylation.

EXAMPLE VII

About 30 grams of O-carboxymethyl chitosan (Nova Chem. Limited, Nova Scotia, Canada) having a 180 minute carboxymethylation time is dissolved in about 900 ml of deionized water. About 30 grams of mannitol, a tonicity agent, and about 5 grams pilocarpine are added to the solution. The pH is adjusted to about 4.7 by adding 37% HCl solution. The final volume is adjusted to one liter with deionized water. No autoclaving is performed on the O-carboxymethyl chitosan. Data is presented in TABLE 3. A graphical comparison of autoclaved (EXAMPLE IV) and non-autoclaved (EXAMPLE VII) at 180-minutes carboxymethylation delivery solutions is presented in FIG. 3.

EXAMPLE VIII

An aqueous O-carboxymethyl chitosan/pilocarpine solution is prepared as in Example VII, with the exception being that a 20-minute-carboxymethylated chitosan is used. Data is presented in TABLE 3. A graphical comparison of autoclaved (EXAMPLE V) and non-autoclaved (EXAMPLE VIII) at 20 minutes carboxymethylation delivery solutions is presented in FIG. 4.

TABLE 3

Time (minutes) from instillation in eye	0.5% pilocarpine in 3% of **20-min**-carboxymethylated O-carboxymethyl chitosan **- no autoclaving -**	0.5% pilocarpine in 3% of **180-min**-carboxymethylated O-carboxymethyl chitosan **- no autoclaving -**
0	0.0	0.0
25	27.8	34.5
40	34.0	38.8
60	32.0	31.2
120	23.7	22.2
180	13.4	11.7
240	3.1	4.2

A comparison of EXAMPLES IV and V with EXAMPLES VII and VIII, respectively, and an examination of FIGS. 3 and 4 reveal that autoclaving improves the effectiveness of O-carboxymethyl chitosan for highly carboxymethylated chitosan (180-min. carboxymethylation period) but reduces effectiveness for low carboxymethylated chitosan (20-minutes carboxymethylation period).

The invention has been described in detail, with reference to certain preferred embodiments, in order to enable the reader to practice the invention without undue experimentation. However, a person having ordinary skill in the art will readily recognize that many of the components and parameters may be varied or modified to a certain extent without departing from the scope and spirit of the invention. Furthermore, titles, headings, or the like are provided to enhance the reader's comprehension of this document, and should not be read as limiting the scope of the present invention. Accordingly, the intellectual property rights to this invention are defined only by the following claims, and reasonable extensions thereof.

CLAIMS

1. An ophthalmic composition, comprising:
   (a) about 0.1 to 25 weight percent O-carboxyalkyl chitosan;
   (b) about 0.01 to 10 weight percent of an ophthalmic delivery agent; and
   (c) an ophthalmic carrier.

2. An ophthalmic composition of claim 1, wherein said ophthalmic composition has a pH of about 4 or higher.

3. An ophthalmic composition of claim 1, wherein said O-carboxyalkyl chitosan is O-carboxymethyl chitosan.

4. An ophthalmic composition of claim 1, comprising 1 to 10 weight percent O-carboxymethyl chitosan.

5. An ophthalmic composition of claim 1, comprising:
   (a) about 1 to 6 weight percent O-carboxyalkyl chitosan;
   (b) about 0.01 to 2 weight percent of an ophthalmic delivery agent; and
   (c) about 98.99 to 93.99 weight percent ophthalmic carrier.

6. An ophthalmic composition of claim 1, wherein said delivery agent is a pH-sensitive delivery agent.

7. An ophthalmic composition of claim 1, wherein said delivery agent is selected from the group consisting of 3H-thymidine, acetylcholine chloride, acyclovir, adrenaline, amethocaine, aminocaproic acid, antazoline phosphate, arachidonic acid, atropine, benoxinate hydrochloride, betaxolol hydrochloride, bupivacaine, carbachol, carteolol, chloramphenicol, chlortetracycline hydrochloride, chymatrypsin, clonidine, cocaine, corynanthine, cromolyn sodium, cyclopentolate, demecarium bromide, dexamethasone, dibutoline, dichlorphenamide, diclofenac, dipivefrin hydrochloride, echodtiophate iodide, ephedrine, epinephrine bitartrate, erythromycin, ethambutol, etidocaine, eucatropine, fluoromethalone, fluorometholone, gentamicin sulfate, gramicidine, H-thymidine, homatropine hydrobromide, hyaluronic acid, hydrocortisone, idoxuridine, indomethacin, inositol triphosphate, inositol phosphates, isoflurophate, isosorbide, lachesine, levobunolol, levocabastine, lidocaine, lignocaine, medrysone, mepivacaine, methacholine,

methazolamide, naphazoline hydrochloride, natamycin, neomycin sulfate, neostigmine, noradrenaline, ofloxacin, oxybuprocaine, oxymetazolin, oxyphenonium, pheniramine maleate, phenylephrine hydrochloride, phosphatidylinositol phosphates, physostigmine, pilocarpine hydrochloride, polyhexamethylene biguanides, polymyxin B sulfates, prednisolone sodium phosphate, proparacaine hydrochloride, proxymethacaine, pyrilamine maleate, scopolamine hydrobromide, sorbinil, sulfacetamide, sulfisoxazole disolamine, tamoxifen, tetracaine hydrochloride, tetracycline, tetrahydrozoline hydrochloride, timolol maleate and hemihydrate, trifluridine, tropicamide, vidarabine, and other ophthalmically acceptable salts thereof and mixtures thereof.

8. An ophthalmic composition of claim 1, wherein said delivery agent is selected from the group consisting of pilocarpine, epinephrine, dipivefrin, hydralazine, carbachol, ophthalmically acceptable salts thereof and mixtures thereof.

9. An ophthalmic composition of claim 9, wherein said delivery agent is hydralazine or an ophthalmically acceptable salt thereof.

10. An ophthalmic composition of claim 1, wherein said composition has a viscosity of 50 to 500 centipoise.

11. An ophthalmic composition of claim 1, wherein said composition is an artificial tears fluid.

12. An ophthalmic composition of claim 1, wherein said composition has a viscosity of 25,000 to 200,000 centipoise.

13. An ophthalmic composition of claim 1, comprising about 1 to 6 weight percent O-carboxymethyl chitosan.

14. An ophthalmic composition of claim 1, comprising:

(a) about 1 to 6 weight percent O-carboxymethyl chitosan;

(b) about 0.01 to 2 weight percent of an ophthalmic delivery agent; and

(c) about 98.99 to 93.99 weight percent ophthalmic carrier.

15. An ophthalmic composition of claim 14, wherein said delivery agent is selected from the group consisting of pilocarpine, epinephrine, dipivefrin, hydralazine, carbachol, ophthalmi-

cally acceptable salts thereof and mixtures thereof; and wherein the pH of said ophthalmic composition is above about 4.

16. An ophthalmic composition of claim 1, wherein said O-carboxymethyl chitosan has been subjected to autoclaving at elevated temperatures for a predetermined time period.

17. An ophthalmic composition of claim 16, wherein said O-carboxymethyl chitosan has been subjected to autoclaving at temperatures of 100 to 150°C for a time of 5 to 60 minutes.

18. A method of delivering an agent to the ocular environment, comprising the steps of:

(a) providing an ophthalmic composition including O-carboxyalkyl chitosan at an acidic pH of above about 4;

(b) dispensing said ophthalmic composition to the ocular environment through a means for altering the pH, thereby altering the pH to an ocularly acceptable pH.

19. A method of claim 18, wherein said ocularly acceptable pH is about 6 to 8.

20. A method of claim 18, wherein said acidic pH is about 4 to 6.

21. A method of claim 18, wherein said chitosan is O-carboxymethyl chitosan.

22. An ophthalmic dispenser, comprising:

(a) a container defining a reservoir and having an outlet;

(b) an ophthalmic composition. including O-carboxymethyl chitosan at a pH of about 4 to 6, retained within said reservoir; and

(c) pH-altering means for increasing the pH of said composition, said pH-altering means being positioned in fluid communication between said solution and said dispenser outlet.

23. An ophthalmic dispenser of claim 22, wherein said ophthalmic composition further includes a pH-sensitive delivery agent.

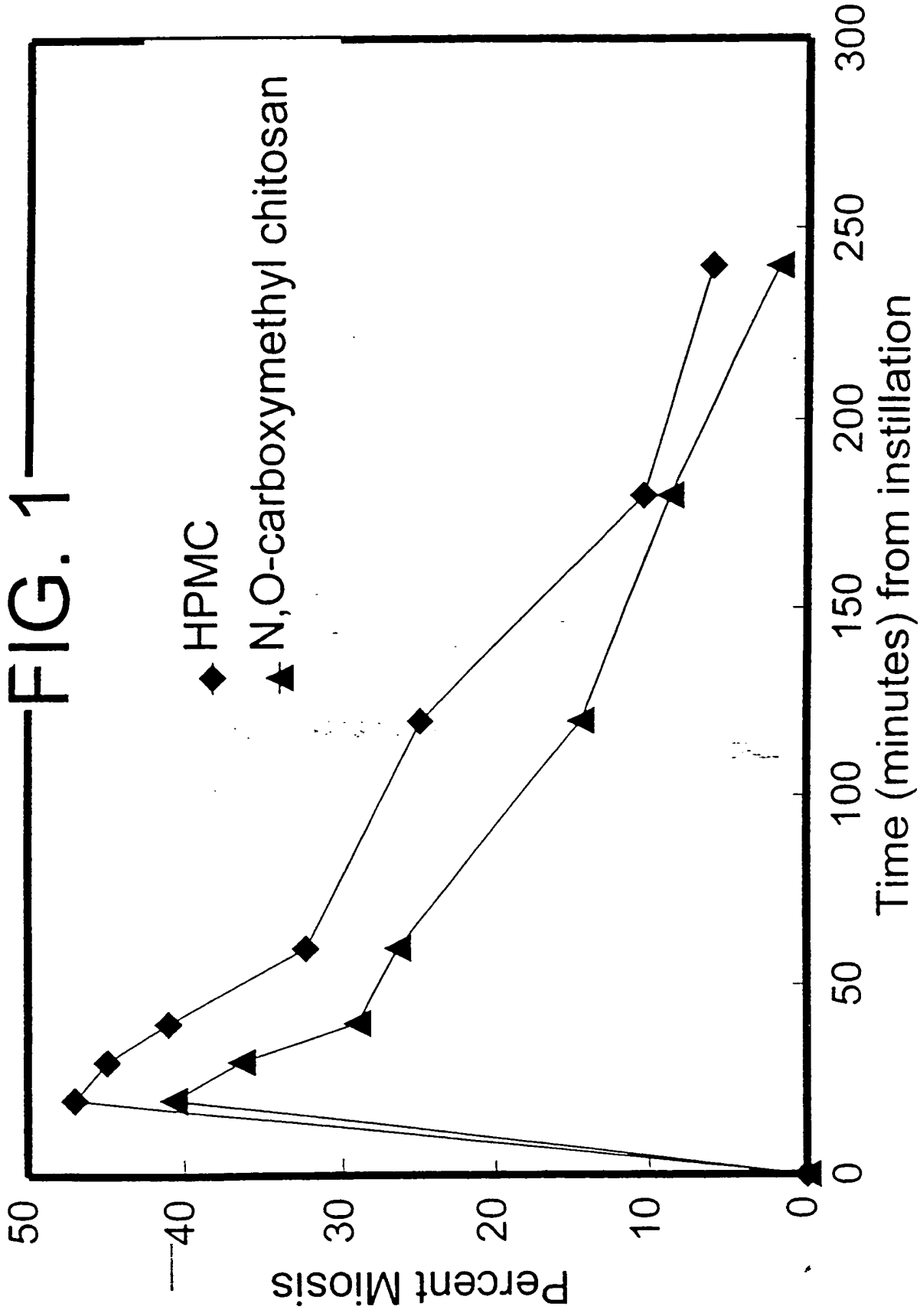
24. An ophthalmic dispenser of claim 23, wherein said pH-sensitive delivery agent is selected from the group consisting of pilocarpine, epinephrine, dipivefrin, hydralazine, carbachol, ophthalmically acceptable salts thereof and mixtures thereof.

25. A method of increasing the bioavailability of a composition including a delivery agent and O-carboxyalkyl chitosan, comprising the step of autoclaving said composition at elevated temperature for a period of time sufficient to increase the retention-enhancing characteristics of the composition.

26. A method of claim 25, wherein said autoclaving is at a temperature of 100 to 150°C for a time of 5 to 60 minutes.

27. A method of claim 25, wherein said method increases the ocular bioavailability of said composition.

FIG. 1


HPMC
N,O-carboxymethyl chitosan
Percent Miosis
Time (minutes) from instillation
0
10
20
30
40
50
0
50
100
150
200
250
300

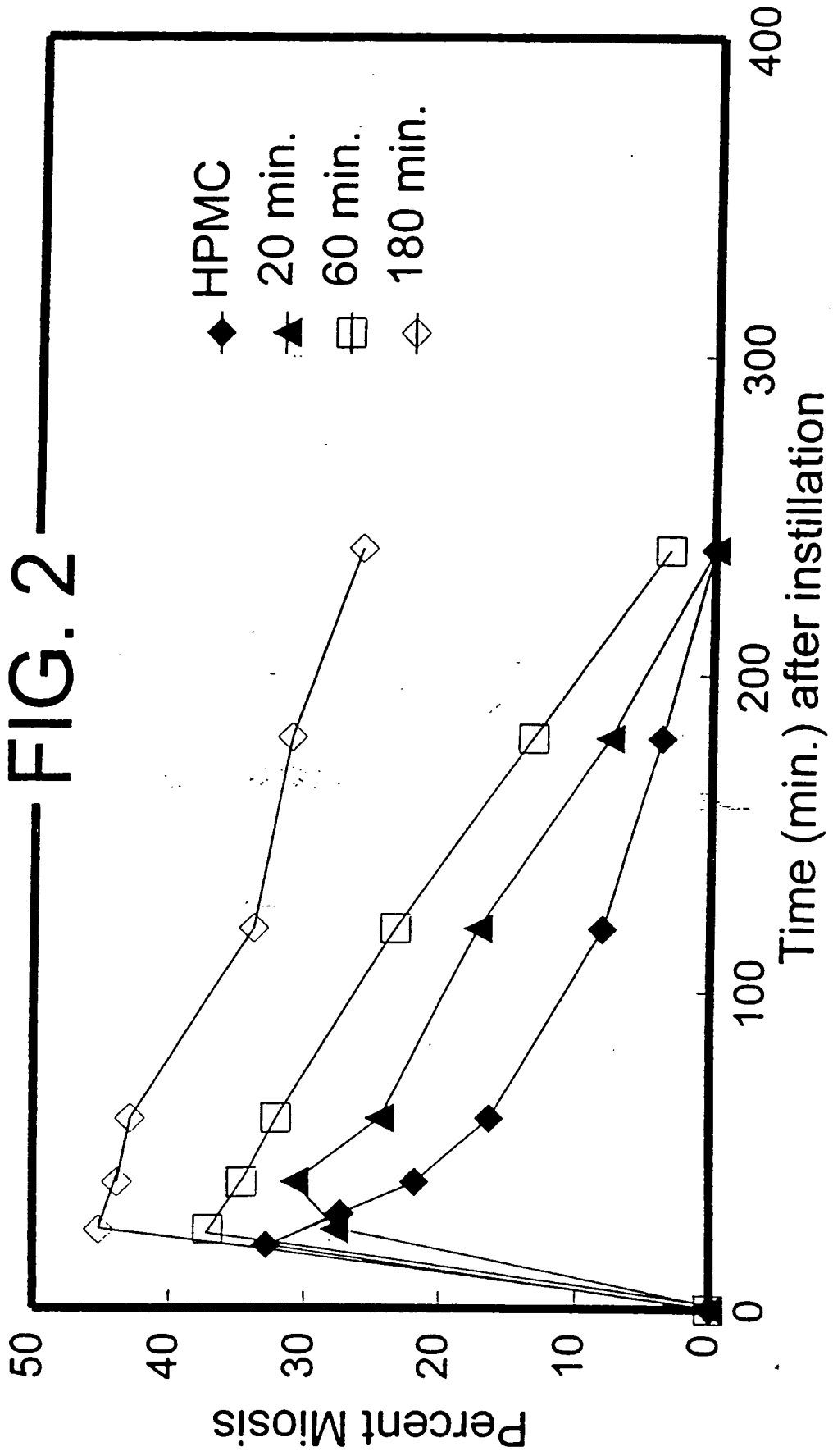

HPMC
20 min.
60 min.
180 min.
Percent Miosis
0
10
20
30
40
50
0
100
200
300
400
Time (min.) after instillation

FIG. 2

FIG. 3

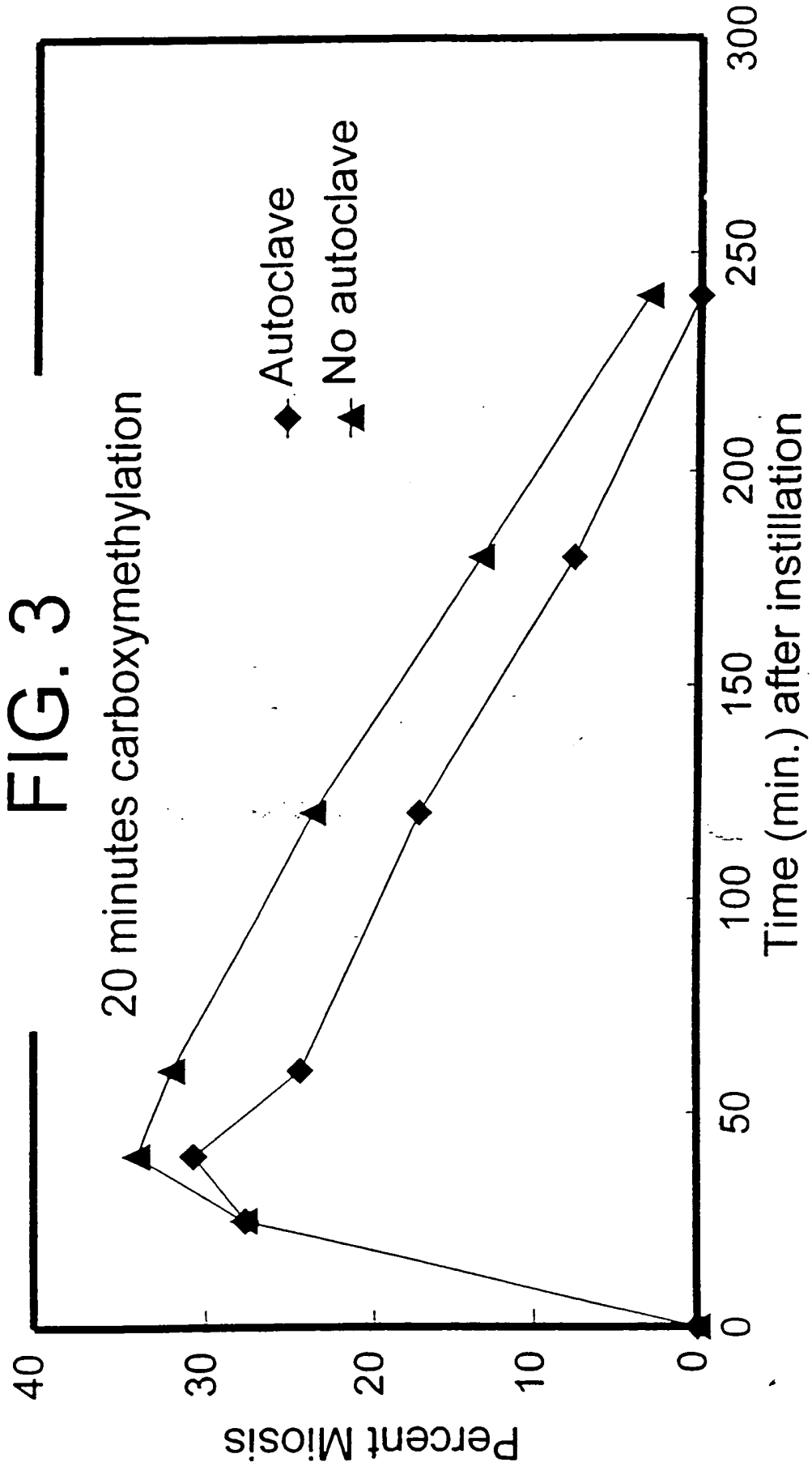
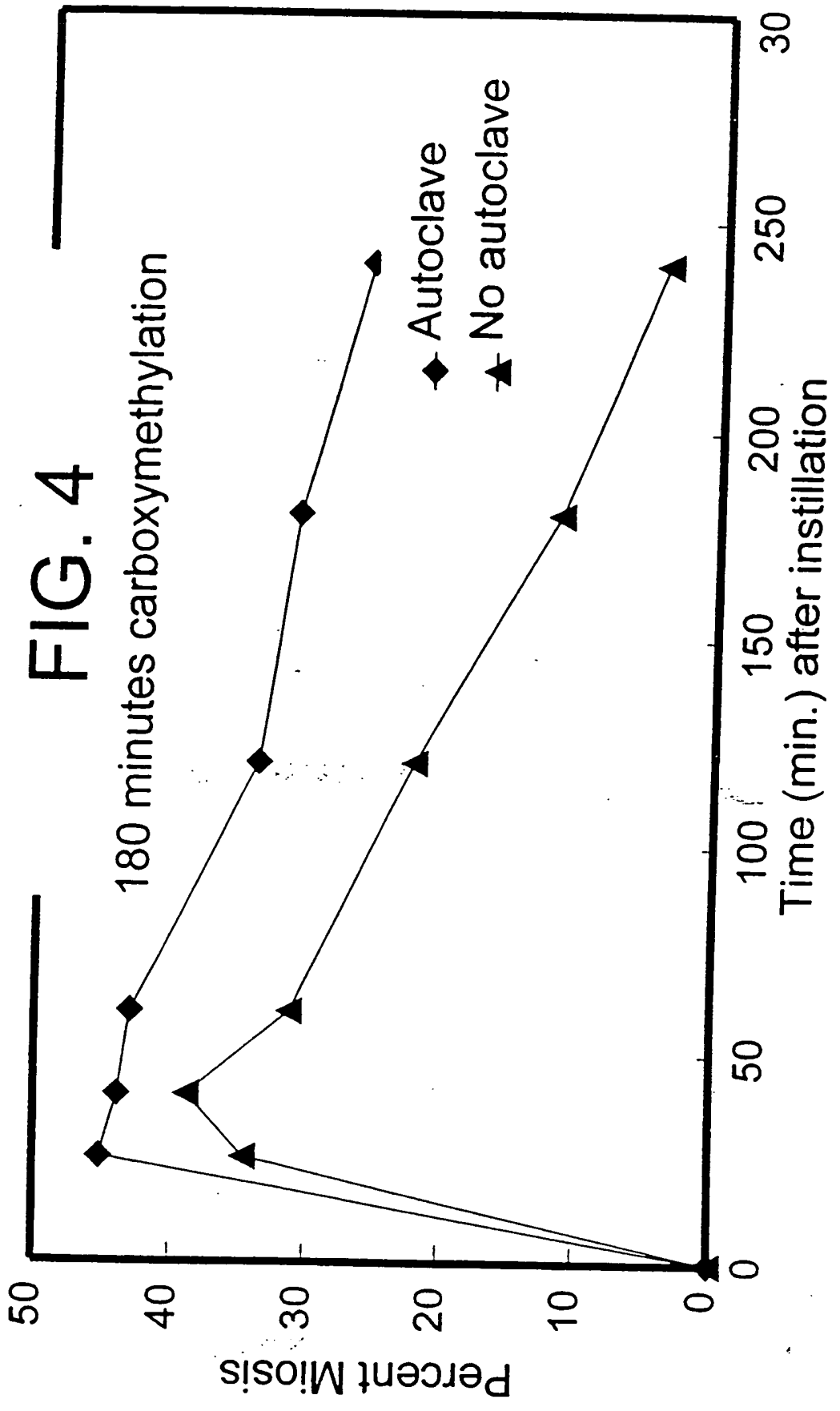

20 minutes carboxymethylation
Autoclave
No autoclave
Percent Miosis
0
10
20
30
40
0
50
100
150
200
250
300
Time (min.) after instillation

FIG. 4


180 minutes carboxymethylation
Autoclave
No autoclave
Percent Miosis
0
10
20
30
40
50
0
50
100
150
200
250
300
Time (min.) after instillation

INTERNATIONAL SEARCH REPORT

Inter nal Application No
PC1/EP 96/03477

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K9/08 A61K47/36

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CHEMICAL ABSTRACTS, vol. 113, no. 19, 5 November 1990 Columbus, Ohio, US; abstract no. 165382, BIAGINI G. ET AL: "N-Carboxymethyl chitosan induces neovascularization" XP002019227 see abstract	1
A	& SKJAAK-BRAEK G. ET AL: "Chitin Chitosan : Sources, Chem., Biochem., Phys. Prop., Aplic. (Proc. Int. Conf.) 4th" 1989 , ELSEVIER , LONDON see page 671 - page 677 ---	1
A	US 5 422 116 A (SHAU-FONG Y. ET AL) 6 June 1995 see claim 1 ---	1

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
22 November 1996	06. 12. 96

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl, Fax (+ 31-70) 340-3016	Boulois, D

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INTERNATIONAL SEARCH REPORT

Interr nal Application No
PC1/EP 96/03477

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	EP 0 665 022 A (PRODEX INC) 2 August 1995 see page 3; example 1	1
A	EP 0 342 557 A (FIDIA SPA) 23 November 1989 see page 18, line 51 - line 55	1
A	EP 0 249 779 A (ETABLISSEMENTS TEXCONTOR) 23 December 1987 see claim 2	1
A	EP 0 426 368 A (PFIZER HOSPITAL PRODUCTS GROUP INC.) 8 May 1991 cited in the application see claim 1	1

INTERNATIONAL SEARCH REPORT

Interna al application No.
PCT/EP 96/03477

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claim(s) 18-21 is(are) directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

2. [ ] Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

information on patent family members

International Application No
PC[illegible]/EP 96/03477

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
US-A-5422116	06-06-95	AU-A-	1464995	04-09-95
		CA-A-	2181715	24-08-95
		WO-A-	9522315	24-08-95
		NO-A-	963431	09-10-96
		ZA-A-	9501323	18-08-95
EP-A-665022	02-08-95	NONE		
EP-A-342557	23-11-89	AT-T-	114322	15-12-94
		AU-B-	629551	08-10-92
		AU-A-	3571889	29-11-89
		CA-A-	1336087	27-06-95
		DE-D-	68919435	05-01-95
		DE-T-	68919435	06-07-95
		WO-A-	8910940	16-11-89
		EP-A-	0615979	21-09-94
		ES-T-	2063779	16-01-95
		IL-A-	90273	27-11-95
		JP-T-	2504164	29-11-90
		US-A-	5122598	16-06-92
		US-A-	5466461	14-11-95
EP-A-249779	23-12-87	DE-A-	3775833	20-02-92
		US-A-	4826826	02-05-89
EP-A-426368	08-05-91	AT-T-	116555	15-01-95
		AU-A-	612085	27-06-91
		CA-A-	2028709	01-05-91
		DE-D-	69015775	16-02-95
		DE-T-	69015775	11-05-95
		ES-T-	2066152	01-03-95
		IE-B-	64988	20-09-95
		JP-A-	3167201	19-07-91
		JP-B-	7090041	04-10-95
		US-A-	5093319	03-03-92

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